PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
CLJ/VB60639		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/014770	21.12.2004	23.12.2003

International Patent Classification (IPC) or national classification and IPC
INV. C12N1/21 C12N15/31 C12N15/62 C12N15/63 C07K14/22 C07K16/12 C07K14/315 A61K39/095

Applicant
GLAXOSMITHKLINE BIOLOGICALS S.A. et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27.02.2006	04.04.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/014770

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-73 as originally filed

Sequence listings part of the description, Pages

1-15 received on 01.06.2005 with letter of 24.05.2005

Claims, Numbers

1-60 received on 06.03.2006 with letter of 27.02.2006

Drawings, Sheets

1/16-16/16 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 59 (with respect to industrial applicability)

because:

☒ the said international application, or the said claims Nos. 59 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

☐ no international search report has been established for the said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has, within the applicable time limit:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ neither restricted the claims nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-10 (complete);48-52,54-60 (in part) .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10,48-52,54-60
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-10,48-52,54-60
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10,48-52,54-58,60
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in electronic form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

1 Cited Documents

Reference is made to the following documents:

D1: BRAUN MARTIN ET AL: "Imp/OstA is required for cell envelope biogenesis in Escherichia coli" MOLECULAR MICROBIOLOGY, vol. 45, no. 5, September 2002, pages 1289-1302

D2: GENEVROIS STEPHANIE ET AL: "The Omp85 protein of Neisseria meningitidis is required for lipid export to the outer membrane." EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 22, no. 8, 15 April 2003, pages 1780-1789

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 59 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of said claim (Article 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

2 Unity of Invention (Rule 13.1 PCT)

This Authority considers, that there are 2 inventions covered by the claims, indicated as follows:

Invention 1: claims 1-10 (complete); claims 48-52, 54-60 (in part)
directed to a Neisserial bacterium in which the expression of Imp is functionally downregulated such that the level of LPS in the outer membrane is decreased

Invention 2: claims 11-47, 53 (complete); claims 48-52, 54-60 (in part)
directed to a chimeric protein comprising Imp from a Neisserial strain and having a

disruption in transporting LPS to the outer membrane

Document D2 discloses a Neisserial strain in which Omp85 is downregulated such that the outer membrane lacks LPS and phospholipids. The document shows, that Omp85 is essential for Neisserial viability (cf. abstract).

It follows that the technical feature of claims 1-10 which makes a contribution over said prior art and thus can be considered as a special technical feature within the meaning of Rule 13.2 PCT is the functional downregulation of Imp.

The problem solved by this special technical feature can therefore be construed as the provision of a viable Neisserial strain in which the level of LPS in the outer membrane is decreased.

Claims 11-47 and 53 do not share the above technical feature. A protein whose function is impaired is not equatable with the functional downregulation of the protein, as a protein can only be functionally downregulated in a host, but not *per se.*

In view of the above, the problem and corresponding solutions of the present application can be summarized as follows:

Problem 1: providing a viable Neisserial strain in which the level of LPS in the outer membrane is decreased

Solution 1: a Neisserial strain in which Imp is functionally downregulated

Problem 2: providing a chimeric protein

Solution 2: a chimeric protein comprising a part which is derived from the Imp protein from a Neisserial strain

The ISA considers, that due to the essential difference between problems 1 and 2 and due to the fact, that no other technical feature could be regarded as special technical feature, there is no single inventive concept underlying the claimed inventions. Consequently there

is a lack of unity (Rule 13.1 PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

3 Novelty (Article 33(2) PCT)

In view of the prior art cited, claims 1-10, 48-52 and 54-60 appear to be novel (Article 33(2) PCT), as none of the documents discloses a Neisserial bacterium in which Imp expression is functionally downregulated.

4 Inventive Step (Article 33(3) PCT)

Moreover, the subject-matter of claims 1-10, 48-52 and 54-60 is considered to involve an inventive step in the sense of Article 33(3) PCT.

4.1 D2 represents the closest prior art and discloses a Neisserial strain in which Omp85 is downregulated such that LPS and phospholipids are not localized to the outer membrane, but accumulate in the inner membrane, whereas localization of the outer membrane proteins PorA and Opa is not affected. The document teaches, that Omp85 is essential for Neisserial viability (cf. abstract).

4.2 The objective technical problem to be solved by the present application is thus the provision of a viable Neisserial bacterium in which the expression of a protein involved in LPS transport to the outer membrane is functionally downregulated.

4.3 The solution, namely the downregulation of Imp, is neither disclosed nor rendered obvious from the prior art cited. D2 does not mention Imp. D1 discloses a conditional *E. coli* Imp null mutant, displaying inner and outer membrane disruption and mislocalisation of proteins and lipids. The document shows, that Imp is essential for *E. coli* viability. Due to the non-viability of the *E. coli* Imp null mutant, D1 does not conclusively demonstrate the function of Imp, but only suggests, that Imp is involved

in LPS transport. D3 teaches, that *Neisseria meningitidis* is viable without LPS. However, the LPS-deficient Neisseria mutant has been obtained by downregulation of a protein which is involved in lipid A biosynthesis (LpxA) and not in LPS transport. In view of D1, D2 and D3, it would not have been obvious for the skilled person to downregulate the Imp protein in order to obtain a viable Neisserial bacterium in which the LPS level in the outer membrane is decreased. The subject-matter of claims 1-10, 48-52 and 54-60 is therefore considered inventive (Article 33(3) PCT).

Claims as amended

1. A Neisserial bacterium in which the expression of an Imp protein is functionally downregulated such that the level of LPS in the outer membrane is decreased compared to wild-type Neisserial bacterium.

2. The Neisserial bacterium of claim 1 wherein the Imp expression is functionally downregulated by downregulating expression from an *imp* gene.

3. The Neisserial bacterium of claim 1 or 2 wherein the Imp expression is functionally downregulated by disrupting the structure of the Imp protein.

4. The Neisserial bacterium of claim 3 wherein at least one of the extracellular loops of the Imp protein is disrupted by inserting a sequence from a different protein into the loop to make a chimeric protein.

5. The Neisserial bacterium of claim 3 or 4 wherein the structure of the Imp protein is disrupted by removing part of the sequence of the Imp protein and optionally replacing it with a sequence from a different protein to make a chimeric protein.

6. The Neisserial bacterium of claim 5 wherein at least part of at least one extracellular loop of the Imp protein is removed and optionally replaced with a sequence from a different protein to make a chimeric protein

7. The Neisserial bacterium of any preceding claim, in which the expression of an MsbA protein is functionally downregulated such that the level of LPS in the outer membrane is decreased compared to wild-type Neisserial bacterium.

8. The Neisserial bacterium of claim 7 wherein the MsbA expression is functionally downregulated by downregulating expression from an msbA gene.

9. The Neisserial bacterium of claim 7 or 8 wherein the MsbA expression is functionally downregulated by disrupting the structure of the MsbA protein

10. The Neisserial bacterium of any one of claims 1-9 wherein the bacterium is *Neisseria meningitidis.*

11. A chimeric protein comprising at least one part which is derived from an Imp protein from a Neisserial strain and at least one part which is derived from at least one different protein, wherein the chimeric protein has a disruption in transporting LPS to the outer membrane.

12. The chimeric protein of claim 11 wherein at least one part derived from at least one different protein is inserted into at least one extracellular loop of Imp.

13. The chimeric protein of claim 12 wherein at least a portion of at least one extracellular loop from Imp is deleted and replaced with at least one part derived from at least one different protein.

14. The chimeric protein of claim 11 comprising at least one extracellular loop from an Imp protein linked to a polypeptide sequence from at least one different protein.

15. The chimeric protein of any one of claims 11-14 wherein the Imp protein is from *N. meningitidis.*

16. The chimeric protein of any one of claims 11-15 wherein the Imp protein part of the chimeric protein has a sequence sharing at least 80% identity with the corresponding sequence of SEQ ID No 1.

17. The chimeric protein of any one of claims 11-16 which has a sequence sharing at least 60% identity with the sequence of SEQ ID No. 1.

18. The chimeric protein of any one of claims 11-17 wherein the part derived from a different protein comprises an epitope capable of generating an immune response against a Neisserial protein.

19. The chimeric protein of any one of claims 11-18 wherein the chimeric protein has impaired LPS transporter function compared to the LPS transporter function of a wild-type Imp protein from which it is derived.

20. The chimeric protein of any one of claims 11-19 wherein the part derived from a different protein is inserted into loop 3 of Imp and optionally at least part of the loop 3 is deleted.

21. The chimeric protein of any one of claims 11-20 wherein an Imp sequence corresponding to amino acids 357-416 or a portion thereof of SEQ ID No. 1 is deleted and optionally replaced with a part derived from a different protein.

22. The chimeric protein of any one of claims 11-21 wherein the part derived from a different protein is inserted into loop 8 of Imp and optionally at least part of the loop is deleted.

23. The chimeric protein of any one of claims 11-22 wherein an Imp sequence corresponding to amino acids 648-697 or a portion thereof of SEQ ID No. 1 is deleted and optionally replaced with a part derived from a different protein.

24. The chimeric protein of any one of claims 11-23 wherein the part derived from a different protein is inserted into loop 6 of Imp and optionally at least part of the loop is deleted.

25. The chimeric protein of any one of claims 11-24 wherein an Imp sequence corresponding to amino acids 537-576 or portion thereof of SEQ ID No. 1 is deleted and optionally replaced with a part derived from a different protein.

26. The chimeric protein of any one of claims 11-25 wherein the part derived from a different protein is inserted into loop 2 of Imp and optionally at least part of the loop is deleted.

27. The chimeric protein of any one of claims 11-26 wherein an Imp sequence corresponding to amino acids 295-332 or portion thereof of SEQ ID No. 1 is deleted and optionally replaced with a part derived from a different protein.

28. The chimeric protein of any one of claims 11-27 wherein the part derived from a different protein is inserted into loop 1 of Imp and optionally at least part of the loop is deleted.

29. The chimeric protein of any one of claims 11-28 wherein an Imp sequence corresponding to amino acids 252-271 or portion thereof of SEQ ID No. 1 is deleted and optionally replaced with a part derived from a different protein.

30. The chimeric protein of any one of claims 11-29 wherein the part derived from a different protein is inserted into loop 5 of Imp and optionally at least part of the loop is deleted.

31. The chimeric protein of any one of claims 11-30 wherein an Imp sequence corresponding to amino acids 482-501 or portion thereof of SEQ ID No. 1 is deleted and optionally replaced with an insert part derived from a different protein.

32. The chimeric protein of any one of claims 11-31 wherein the part derived from a different protein is inserted into loop 9 of Imp and optionally at least part of the loop is deleted.

33. The chimeric protein of any one of claims 11-32 wherein an Imp sequence corresponding to amino acids 721-740 or portion thereof of SEQ ID No. 1 is deleted and optionally replaced with a part derived from a different protein.

34. The chimeric protein of any one of claims 11-33 wherein at least one part derived from a different protein is an *N. meningitidis* protein.

35. The chimeric protein of claim 34 wherein at least one part is derived from PorA.

36. The chimeric protein of claim 35 wherein 2 or more parts are derived from 2 or more PorA proteins from different serosubtypes of N. meningitidis.

37. The chimeric protein of any one of claims 34-36 wherein at least one part is derived from Hsf.

38. The chimeric protein of any one of claims 34-37 wherein at least one part is derived from TbpA.

39. The chimeric protein of any one of claims 34-38 wherein at least one part is derived from TbpA –high molecular weight and at least one different part is derived from TbpA – low molecular weight.

40. The chimeric protein of any one of claims 34-39 wherein at least one insert part is derived from NspA .

41. The chimeric protein of any one of claims 34-40 wherein at least one part is a peptide mimotope of a Neisserial LOS.

42. The chimeric protein of any one of claims 34-41 wherein at least one part is derived from Hap.

43. The chimeric protein of any one of claims 11-42 wherein at least one part is derived from a S. pneumoniae protein.

44. The chimeric protein of any one of claims 11-43 wherein at least one part derived from a different protein is surface exposed in the bacterial strain from which it is derived.

45. A polynucleotide comprising a sequence encoding the chimeric protein of any one of claims 11-44.

46. An expression vector comprising the polynucleotide of claim 45.

47. A host cell comprising the expression vector of claim 46.

48. An outer membrane vesicle preparation derived from the bacterium of any one of claims 1-10 or the host cell of claim 47 or comprising the chimeric protein of any one of claims 11-44.

49. The outer membrane vesicle preparation of claim 48 derived from *N. meningitidis* wherein the amount of LPS in the outer membrane vesicle is reduced compared to the amount of LPS in an outer membrane vesicle preparation derived from a strain of *N. meningitidis* where Imp or MsbA is not functionally disrupted.

50. The outer membrane vesicle preparation of any one of claim 48 or 49 wherein the level of LPS is sufficiently low so that the toxicity is reduced to a level at which the outer membrane vesicle preparation has an acceptable level of reactogenicity when inoculated into a patient.

51. The outer membrane vesicle preparation of any one of claims 48-50 wherein LPS present in the outer membrane vesicles is intra-vesicle cross-linked to outer membrane proteins in the outer membrane vesicle.

52. The outer membrane vesicle preparation of any one of claims 48-51 wherein the concentration of lipoproteins in the outer membrane vesicles is equivalent to the concentration of lipoproteins from outer membrane vesicles derived from a non-detergent extraction process.

53. A method for producing the chimeric protein of any one of claims 11-44 comprising the steps of culturing the host cell of claim 47 under conditions under which the chimeric protein is expressed and recovering the expressed chimeric protein.

54. A method for producing the outer membrane vesicle preparation of claim 48-52 comprising the step of culturing the host cell of claim 47 or the bacterium of claims 1-10.

55. A pharmaceutical composition comprising the bacterium of claims 1-10 or a fraction or membrane thereof, the chimeric protein of any one of claims 11-44 or the outer membrane vesicle preparation of any one of claims 48-52, and a pharmaceutically acceptable carrier.

56. The pharmaceutical composition of claim 55 in the form of a vaccine.

57. The pharmaceutical composition of claim 55 or claim 56 further comprising one or more bacterial capsular polysaccharides or oligosaccharides.

58. The pharmaceutical composition of claim 57 wherein the one or more capsular polysaccharides or oligosaccharides is derived from bacteria selected from the group consisting of N. meningitidis serogroup A, C, Y and/or W-135, Haemophilus influenzae b, Streptococcus pneumoniae, and are preferably conjugated to a source of T-helper epitopes.

59. A method of preventing or treating a Neisserial infection by administering the chimeric protein of any one of claims 11-44 or the outer membrane vesicle preparation of any one of claims 48-52 or the pharmaceutical composition of any one of claims 55-58 to a patient in need thereof.

60. A use of the bacterium of any one of claims 1-10 or a fraction or membrane thereof, the chimeric protein of any one of claims 11-44 or the outer membrane vesicle preparation of any one of claims 48-52 in the preparation of a medicament for treatment or prevention of a Neisserial infection.